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(54) POLYPEPTIDE INDUISANT DES ANTICORPS NEUTRALISANT LE VIH
(54) POLYPEPTIDE INDUCING HIV-NEUTRALISING ANTIBODIES

(57)
The invention concerns a polypeptide capable of forming a structure corresponding or analogous to the intermediate state of gp41 and its use in a vaccine for preventing and treating HIV-mediated infections.

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(54) Title: POLYPEPTIDE INDUCING HIV-NEUTRALISING ANTIBODIES

(54) Titre : POLYPEPTIDE INDUISANT DES ANTICORPS NEUTRALISANT LE VIH

(57) Abstract: The invention concerns a polypeptide capable of forming a structure corresponding or analogous to the intermediate state of gp41 and its use in a vaccine for preventing and treating HIV-mediated infections.

(57) Abrégé : La présente invention concerne un polypeptide capable de former une structure correspondant à ou mimant l'état intermédiaire de la gp41 ainsi que son utilisation dans un vaccin utilisation dans la prévention et le traitement des infections par le VIH.

POLYPEPTIDE INDUCING HIV-NEUTRALISING ANTIBODIES

The present invention relates to a mutated polypeptide which derives from the gp41 protein, and also to a vaccine comprising it.

The development of a method for immunizing against HIV is, today, one of the priorities of scientific research.

The major obstacles represented by the great genetic variability of the virus and the poor exposure, to the immune system, of neutralizing viral epitopes considerably slow down the production of neutralizing immunity.

The HIV envelope glycoprotein which is required in order to confer on the virus its infectious nature represents the target for neutralizing antibodies. These characteristics have made the latter a subject of intense investigation.

The glycoprotein envelope (ENV) of the human immunodeficiency virus-1 (HIV-1) is synthesized from the gp160 precursor which, under the action of a protease, gives the gp120 and gp41 subunits.

The attachment of gp120/gp41 to the cellular receptors (CD4 or a receptor for chemokines, such as CCR5 or CXCR-4) induces a change of conformation of gp41 from a latent state (nonfusogenic) to a fusion-active state (fusogenic). Between these two states, a transient, termed "intermediate", state exists, during which gp41 has the appearance of a trans-membrane protein in both the viral and cell membranes (Weissenhorn et al Nature (1997), 387 (6631), 426-30).

Binding experiments have made it possible to establish that the nonfusogenic latent state is characterized by the inaccessibility of large portions of the ectodomaine of gp41. gp120 in fact interacts in such a way as to mask the epitopes. It has, moreover, been shown that the inhibition of the structural change

from the intermediate state toward the fusogenic state by peptides used as competitors can affect viral infection (Weissenhorn W. et al, Molecular Membrane Biology, 1999, 16, 3-9).

The use of the fusogenic state of gp41 for immunization purposes is disclosed in WO 00/40616. According to that application, N-helices can be used alone or in combination with C-helices in order to reproduce, in the latter case, the fusogenic conformation of gp41.

The applicant provides a novel immunization antigen which can be used in immunization against HIV. The applicant has, in fact, demonstrated, for the first time, that the intermediate state of gp41 is capable of inducing antibodies which neutralize primary isolates of HIV.

The present invention therefore relates to a polypeptide capable of forming a structure corresponding to or mimicking the intermediate state of gp41.

According to one embodiment, said polypeptide comprises at least one mutation selected from the group consisting of: T35I or L; V49I or L; Q56I or L; I101D or S; I108D and W94D.

According to a particular embodiment, the polypeptide comprises at least one other mutation selected from the group comprising the following mutations: G13A, L, M, I, W or K; Q17A or E; Q18A or E; A24Q, E, S or R; Q28A; T35I or L; V36Q or E; W37S or D; G38A, V, L, I, M or E; Q39A, V, L, I, M or E; K40E, A, V, L, I or M; Q41A, V, L, I, M or E; Q43A, V, L, I, M or E; L47A or D; V49I or L; R51A, N or E; Q56I; C64S; C70S; or L; W94D; D98A, V, L, I, M or K; R99A, N or E; I101D or S; Y104M or E; I108D; Q119A, V, L, I, M, S, N or R; E120A; K121A; E123A; E125A; R153N or A and R173N or A.

According to a preferred embodiment, the polypeptide according to the present invention

comprises the following mutations: T35I; I101D; T35I + Q28I + I101D; T35I + Q28I + I101D + Q119N; I101D + I108D + Q131N + W37A or I101D + I108D + Q142N + L126D; W37A + I101D + I108D + Q119N; or I101D + I108D + Q119N + L126D.

According to another aspect, the present invention relates to a conjugate comprising a polypeptide according to the invention conjugated to a carrier protein or peptide.

According to another aspect, the present invention relates to a DNA sequence encoding a polypeptide according to the invention or encoding a conjugate according to the invention.

The present invention also relates to an expression vector comprising said DNA sequence, and also to a host cell containing said vector.

A subject of the present invention is also a vaccine against HIV, comprising at least one polypeptide as defined above, at least one conjugate as defined above or at least one expression vector as defined above, a pharmaceutically acceptable support and, optionally, an adjuvant.

Another subject of the present invention relates to the process for preparing a polypeptide as defined above, comprising the expression of said polypeptide using a host cell as defined above.

The invention is described in greater detail in the description which follows.

The phenomenon of conformational change in gp41 preceding the fusion of cell and viral membranes is illustrated in Figure 1.

The applicant has demonstrated, surprisingly, that, in humans, the intermediate state of gp41 induces antibodies which neutralize primary isolates of HIV. Induction of antibodies which neutralize primary isolates can be easily determined using the neutralization assay as described in the article by C. Moog et al (AIDS Research and human retroviruses,

vol. 13(1), 13-27, 1997). In the context of the present invention, it is considered that neutralizing antibodies have been induced by the antigen assayed when the serum diluted to 1/5th causes a 10-fold decrease in the amount of p24 present in the culture supernatant.

In the context of the present invention, the expression "polypeptide corresponding or mimicking the intermediate state of gp41" is intended to mean a polypeptide, preferably a trimeric polypeptide, which, under physiological conditions, has an open conformation. In this open conformation, at least one of the C-helices is not paired around the N-helices in the anti-paralleled orientation as present in the fusogenic form. Preferably, the three C-helices are not paired with the N-helices in the anti-parallel orientation as present in the fusogenic form. In such a conformation, it is probable that the C-helix which is not paired with the central trimer consisting of the N-helices adopts a free ("coil") conformation. In the open conformation according to the invention, the N-helices are paired with one another in the parallel orientation, preferably forming a trimer. In the case of the monomer, in the open conformation according to the invention, the C-helix is not paired with the N-helix.

The production of an open conformation can be demonstrated using the technique of measuring the intrinsic fluorescence of the polypeptide, as described by Schmid, F.X. (1989) "Spectral methods of characterising protein conformation and conformational changes" Creighton, T.E. Protein structure- a practical approach. pp251-284, IRL, Oxford University Press. In summary, the polypeptide will be excited at 295 nm and the fluorescence emission spectrum will be recorded at 310-380 nm. There is a correlation between the emission maximum of these wavelengths and the environment of the tryptophans in the structure. A tryptophan residue

which is totally exposed to the solvent (i.e. a hydrophilic environment) has an emission maximum of approximately 355 nm, whereas a tryptophan residue which is protected from the solvent (i.e. present on the inside of the polypeptide) has an emission maximum of approximately 325 nm. In its trimeric form, the polypeptide according to the invention has 9 tryptophan residues at the N/C interfaces. The production of an open conformation will therefore result in an increase in the emission maximum recorded at 310-380 nm.

The polypeptide according to the invention has the particularity of being stable, i.e. it conserves it intermediate conformation under physiological conditions. The stability of the peptide according to the invention can easily be controlled using the differential scanning calorimetry (DSC) technique which is well known to those skilled in the art. Reference may be made, for example, to the articles by A. Cooper et al, Phil Trans. R. Soc. Lon. A (1993) 345, 23-25, and by V.V. Plotnikov et al. Analytical Biochemistry 250, 237-244 (1997).

The applicant has also demonstrated, surprisingly, that the polypeptide according to the invention conserves its "open" conformation in highly acidic medium. This property makes the polypeptide according to the invention an immunization antigen which can be administered orally. The applicant has in fact shown that the ectodomain of the gp14 protein is extremely thermostable at pH 2.5. Measurement of the Tm (temperature at which 50% of the proteins present are denatured) of gp41 in 50 mM of sodium formate by DSC (differential scanning calorimetry) gives a value of 110°C, the start of the denaturation phenomenon appearing at approximately 100°C at pH = 2.5. The thermostability of the gp41 protein at neutral pH has been evaluated by Weissenhorn et al. (EMBO, 1996, 7, 1507-1514), The Tm measured at neutral pH by these authors is 78°C, which means that, surprisingly, this

protein is more stable at acid pH than at neutral pH. These results were confirmed by circular dichroism analysis aimed at calculating the percentage of alpha helix in the protein.

The applicant has also shown that the polypeptide according to the invention has the same particularity. This specific property makes the polypeptide according to the invention an immunization antigen of choice for oral administration.

The polypeptide according to the invention consists of the sequence corresponding to the gp41 protein lacking all or part, preferably all, of the sequence corresponding to the transmembrane domain. The polypeptide according to the invention also lacks all or part, preferably all, of the sequence corresponding to the fusion peptide. According to a preferred embodiment, all or part of the sequence corresponding to the intracytoplasmic domain is also deleted.

In the context of the present invention, the term "gp41" is intended to mean a gp41 protein derived from any strain of HIV1 or of HIV2, preferably of HIV1, including laboratory strains and primary isolates. By way of illustration, mention may be made of MN and BX08 strains.

The nucleotide sequence and peptide sequence of a large number of gp41 proteins are known and available for example, over the internet (http://hivweb.lanl.gov/) and in the corresponding Los Alamos compendia. It is clear that any sequence into which one or more conservative mutations has been introduced is also included in the context of the present invention.

The various constituent domains of gp41, identified above, are defined herein with reference to the sequence of gp41 LAI as represented in Figure 2, in which the 1st amino acid A is numbered 1. Not all authors agree on the definition of the sequences

corresponding to the fusion peptide and to the transmembrane domain. According to some authors, the fusion peptide corresponds to the sequence 1-32.
Although deletion of the sequence 1-23 is preferred, in particular because of the presence of a methionine at position 24, deletion of the sequence 1-32 is also suitable in the context of the present invention.

Regarding the transmembrane domain, some authors consider that the latter begins at residue 154. Although deletion of the sequence 173-194 is preferred, deletion of the sequence 154-194 is also envisaged in the context of the present invention.

The polypeptide according to the invention can be obtained by mutation of the natural sequence of gp41.

According to a preferred embodiment, the polypeptide according to the invention is prepared from the sequence of gp41 LAI in which the transmembrane domain and the fusion peptide, and also part of the intracytoplasmic domain, have been deleted. This sequence is represented in Figure 3.

The mutations identified in the subsequent text are numbered with reference to the sequence in Figure 3, in which the 1st amino acid M is numbered 1.

The applicant has demonstrated a certain number of mutations which destabilize the structure of the latent state and/or of the fusogenic state of gp41 (in the monomeric and/or trimeric state) and/or stabilize the intermediate state of gp41 (in the monomeric and/or trimeric state), and/or promote the transconformation from the latent state to the intermediate state and/or hinder the transconformation from the intermediate state to the fusogenic state. These mutations make it possible to stabilize the polypeptide according to the invention in an open conformation.

The applicant has demonstrated that the polypeptide according to the present invention, and more particularly the N- and C-helices identified in

the structure in the fusogenic state, may be divided into four regions (numbered with reference to the sequence in Figure 3) corresponding, respectively, to the sequences Ala7-Ile25 (region 1), Glu26-Ala44 (region 2), Arg45-Leu58 (region 3) and Trp94-Lys131 (region 4), and that the mutations which modify the electrostatic properties and/or the amphilicity and/or the structural properties of these regions lead to the production of a polypeptide in the open conformation, according to the invention. The applicant has, in particular, shown that modifying the structure of region 2, changing from an extended structure to an α-helical structure, leads to the formation of the N-helix and the production of an open conformation.

The modifications introduced can be evaluated using conventional methods which are well known to those skilled in the art. By way of a method which may be used, mention may be made, for example, of the method of Eisenberg, which allows measurement of the mean-hydrophobicity and of the mean hydrophobic moment along the sequence; and the analysis of hydrophobic clusters, of molecular hydrophobicity potentials or of molecular electrostatic potentials. The structural modifications can be evaluated, for example, with studies of molecular dynamics and also with software for predicting secondary structures, such as PHD, NPSA, etc. These methods are described in the following articles: **Method of Eisenberg:** D., Schwarz, E., Komaromy, M. and Wall, R. 1984 *Journal of Molecular Biology,* 179: 123-142; **Analysis of hydrophobic clusters:** Gaboriaud, C., Bissery, V., Benchetrit, T. and Mornon, JP. 1987, *FEBS Letters.* 224 (1): 149-55; **Molecular Hydrophobicity Potential:** Brasseur, R. 1991, *Journal of biological chemistry,* 266: 16120-16127; **Molecular Electrostatic Potential:** Delleers, M. and Brasseur, R. 1989 *Biochemical Pharmacology,* 38 (15): 2441-2447; **Molecular Dynamics (study of stability):** Berendsen, H.J.C., van der Spoel, D. and van Drunen, R.

1995 GROMACS, *Computer Physics Communications*, 95: 43-56; **Molecular Dynamics (transconformation):** Guilbert, C., Perahia, D. and Mouawad, L. 1995, *Computer Physics Communications*, 91: 263-273; **PHD:** Rost, B. and Sander, C. 1994 *Proteins*, 19: 55-72; **NPSA:** Combet, C., Blanchet, C., Geourjon, C. and Deléage, G. 2000 *Trends in biochemical sciences*, *25 (3): 147-150*.

The applicant has shown that the mutations as defined above which are related to the amino acids of region 1 hinder the interactions of this region with regions 3 and 4 in the latent state and/or with region 4 in the fusogenic state, and/or promote the multimerization of this region in the fusogenic state. The mutations as defined above which relate to the amino acids of region 2 promote an α-helical structure for this region and/or the transconformation to such a structure, and/or hinder the interactions of this region with regions 3 and 4 in the latent state and/or with region 4 in the fusogenic state, and/or promote the multimerization of this region in the fusogenic state. The mutations as defined above which relate to the amino acids of region 3 promote the multimerization in the latent state and/or in the fusogenic state, and/or hinder the interactions of this region with regions 1 and 2 in the latent state and/or with region 4 in the fusogenic state. The mutations as defined above which relate to the amino acids of region 4 promote the multimerization of this region in the latent state, and/or hinder the interactions with this region with regions 1 and 2 in the latent state and/or with regions 1, 2 or 3 in the fusogenic state.

Regions 1 to 4 of the polypeptide according to the invention and also the function of the mutations are summarized in Figures 4 and 5. In Figure 4, regions 1 to 4 are located in the complete gp41 protein which, therefore, includes the fusion peptide (AA 1-23) with the 1st amino acid A numbered 1).

In the following text, the amino acids are represented by their international code, and the code L_1NNL_2 indicates that the amino acid represented by L_1 located at position NN is mutated at the amino acid represented by L_2.

A subject of the present invention is therefore a polypeptide which comprises at least one mutation, preferably two mutations, selected from the group consisting of: T35I or L; V49I or L; Q56I or L; I101D or S; I108D and W94D, preferably selected from the group consisting of: T35I and I101D or S. This first mutation is preferably combined with at least one other mutation, preferably with 1 to 3 mutations, preferably located in one or more of regions 1 to 4. When two mutations are selected from the above group, these mutations are combined with at least one other mutation, preferably with 1 or 2 mutations, preferably located in one or more of regions 1 to 4.

The polypeptide according to the present invention preferably comprises at least two mutations which hinder the interactions between the N/C helices. According to a particularly preferred aspect, these two mutations are combined with at least one mutation, preferably two mutations, which promote the interactions between the N helices in a parallel orientation. The said polypeptide thus obtained may also advantageously comprise one or more of the other mutations having the effects identified in figure 4 and in particular at least one of the mutations identified in table 1 in columns 3 to 6.

These additional mutations are preferably selected from the group comprising the following mutations: G13A, L, M, I, W or K; Q17A or E; Q18A or E; A24Q, E, S, or R; Q28A; T35I or L; V36Q or E; W37S or D; G38A, V, L, I, M or E; Q39A, V, L, I, M or E; K40E, A, V, L, I or M; Q41A, V, L, I, M or E; Q43A, V, L, I, M or E; L47A or D; V49I or L; R41A, N or E; Q56I; C64S; C70S; or L; W94D; D98A, V, L, I, M or K; R99A, N or E;

I101D or S; Y104M or E; I108D; Q119A, V, L, I, M, S, N or R; E120A; K121A; E123A; E125A; R153N or A and R173N or A.

Preferably, positions K40 and D98 are not mutated simultaneously.

By way of example of combinations of additional mutations which can be used in the context of the present invention, mention may be made of: Q17A + Q18A; Q17A + Q18A + Q28A; Q41E + Q43E; C64S + C70S; E120A + K121A; E120A + E123A and K121A + E125A.

Examples of preferred polypeptides according to the present invention are the polypeptides comprising the following mutations: T35I; V49I; Q56I; T35I + Q28I = I101D; T35I + Q28I + I101D + Q119N; V49I + Q28I + I101D; V49I + Q28I + I101D + Q119N; Q56I + Q28I + I101D; Q56I + Q28I + I101D + Q119N; I101D; I101S; I108D; W94D; I101D + Q28I + V49I; I101D + Q28I + Q56I; I101S + Q28I + T35I; I101S + Q28I + V49I; I101S + Q28I + Q56I; I108D + Q28I + T35I; I108D + Q28I + V49I; I108D + Q28I + Q56I; W94D + Q28I + T35I; W94D + Q28I + V49I; W94D + Q28I + Q56I; I101D + I108D + Q131N + W37A; I101S + I108D + Q131N + W37A; W94D + I108D + Q131N + W37A; I101D + W94D + Q131N + W37A; I101S + W94D + Q131N + W37A; I101D + I108D + Q142N + L126D; I101S + I108D + Q142N + L126D; W94D + I108D + Q142N + L126D; I101D + W49D + Q142N + L126D; I101S + W94D + Q142N + L126D; T35I + Q28I + I101S + Q119N; V49I + Q28I + I101S + Q119N; Q56I + Q28I + I101S + Q119N; T35I + Q28I + I108D + Q119N; V49I + Q28I + I108D + Q119N; Q56I + Q28I + I108D + Q119N; T35I + Q28I + W94D + Q119N; V49I + Q28I + W94D + Q119N; Q56I + Q28I + W94D + Q119N; T35L; V49L; Q56L; T35L + Q28I + I101D; T35L + Q28I + I101D + Q119N; V49L + Q28I + I101D; V49L + Q28I + I101D + Q119N; Q56L + Q28I + I101D; Q56L + Q28I + I101D + Q119N; I101D + Q28I + V49L; I101D + Q28I + Q56L; I101S + Q28I + T35L; I101S + Q28I + V49L; I101S + Q28I + Q56L; I108D + Q28I + T35L; I108D + Q28I + V49L; I108D + Q28I + Q56L; W94D + Q28I + T35L; W94D + Q28I + V49L; W94D + Q28I + Q56L;

T35L + Q28I + I101S + Q119N; V49L + Q28I + I101S + Q119N; Q56L + Q28I + I101S + Q119N; T35L + Q28I + I108D + Q119N; V49L + Q28I + I108D + Q119N; Q56L + Q28I + I108D + Q119N; T35L + Q28I + W94D + Q119N; V49L + Q28I + W94D + Q119N; or Q56L + Q28I + W94D + Q119N; W37A + I101D + I108D + Q119N; or I101D + I108D + Q119N + L126D.

These polypeptides preferably correspond to a polypeptide of sequence SEQ ID No. 2 into which the above mutations have been introduced.

Examples of particularly preferred polypeptides in the context of the present invention are the polypeptides of sequence SEQ ID No. 2 into which the mutations: T35I; I101D; T35I + Q28I + I101D; T35I + Q28I + I101D + Q119N; I101D + I108D + Q131N + W37A or I101D + I108D + Q142N + L126D; W37A + I101D + I108D + Q119N; or I101D + I108D + Q119N + L126A have been introduced.

Other modifications may be introduced into the polypeptide of the invention, for example in order to facilitate expression and promote better solubility. Advantageously, one or both cysteine at position 64 and position 69 may be replaced, for example, with serine.

Although the polypeptide according to the invention has an open conformation which is stable under physiological conditions, this conformation can be reinforced by adding cysteine residues to the ends of the polypeptide. To this end, two additional cysteine residues can be added at the N-terminal or at the C-terminal, preferably at the N-terminal, of the polypeptide according to the invention so as to covalently fix the trimer in an open conformation. In this case, the amino acids Q17 and Q18 are preferably mutated to cysteine.

The mutations proposed may be combined in order to obtain a synergistic effect or, at the very least an additive effect. The polypeptide according to the present invention preferably comprises at least two

mutations which hinder the interactions between the N/C helices. According to a particularly preferred aspect, these two mutations are combined with at least one mutation, preferably at least two mutations, which promote the interactions between the N helices in a parallel orientation. The said polypeptide thus obtained may also advantageously comprise one or more of the other mutations having the effects identified in Figure 4 and in particular at least one of the mutations identified in Table 1 in columns 3 to 6. Preferably, positions K40 and D98 are not simultaneously mutated.

The mutations proposed in the context of the present invention are summarized in Table 1.

Table 1

Initial AA/position	Mutation	Functions					
		1	2	3	4	5	6
		Hinder the interactions between the N- and C-helices in the fusogenic state	Promote the interactions between the N-helices in the fusogenic state	Promote an α-helical structure	Hinder the interactions between regions 2 and 3 in the latent state	Hinder a loop structure in region 2	Eliminate a potential area of interaction
G13	A,L,M,I W or K	x		x			
Q17	A or E	x					
Q18	A or E		x				
A24	Q, E, S or R	x		x			
Q28	A		x				
T35	A		x				
V36	Q or E	x		x			
G38	A, V, L, I, E or M	x		x			
K40	E, A, V, L, I or M	x					
D98	A, V, L, I, M or K	x					
Q39	A, V, L, I, M or E					x	
Q41	A, V, L, I, M or E					x	

Initial AA/position	Mutation	Functions					
		1	2	3	4	5	6
		Hinder the interactions between the N- and C-helices in the fusogenic state	Promote the interactions between the N-helices in the fusogenic state	Promote an α-helical structure	Hinder the interactions between regions 2 and 3 in the latent state	Hinder a loop structure in region 2	Eliminate a potential area of interaction
Q43	A, V, L, I, M or E	x				x	
L47	A or D				x		
V49	I or L		x				
R51	A, N or E				x		
Q56	L or I		x				
C64	S						x
C70	S						x
W94	D	x					
D98	A, L, V, I, M or K	x					
R99	A, N or E	x					
I101	D or S	x					
Y104	M or E	x					
I108	D	x					
Q119	A, V, L, I, M, S or R	x					
E120	A						x
K121	A						x
E123	A						x
E125	A						x

Initial AA/position	Mutation	Functions					
		1	2	3	4	5	6
		Hinder the interactions between the N- and C-helices in the fusogenic state	Promote the interactions between the N-helices in the fusogenic state	Promote an α-helical structure	Hinder the interactions between regions 2 and 3 in the latent state	Hinder a loop structure in region 2	Eliminate a potential area of interaction
W37	S or D	x					
R153	N or A						x
R173	N or A						x

The polypeptide according to the invention can be obtained using any conventional technique of chemical synthesis or of genetic engineering.

When the polypeptide is produced by chemical synthesis, the polypeptide according to the invention can be synthesized even in the form of a single sequence or in the form of several sequences which are then attached to one another. The chemical synthesis can be carried out in solid phase or in solution, these two techniques for synthesis being well known to those skilled in the art. These techniques are in particular described by Atherton and Shepard in "solid phase peptide synthesis" (IRL press Oxford, 1989) and by Houbenweyl in "method der organischen chemie" edited by E. Wunsch vol, 15-I and II thieme, Stuttgart, 1974, and also in the following articles: Dawson PE et al (Synthesis of proteins by native chemical ligation Science 1994; 266(5186): 776-9); Kochendoerfer GG et al (Chemical protein synthesis. Curr Opin Chem Biol 1999; 3(6): 665-71); and Dawson PE et al Synthesis of native proteins by chemical ligation, Annu Rev Biochem 2000; 69: 923-60.

The polypeptide according to the invention can also be produced using genetic engineering techniques which are well known to those skilled in the art. These techniques are described in detail in Molecular Cloning: a molecular manual, by Maniatis et al. (Cold Spring Harbor, 1989). Conventionally, the DNA sequence encoding the polypeptide according to the invention is inserted into an expression vector, which is mutated by site-directed mutagenesis. When several mutations must be introduced, a first mutagenesis reaction is carried out and then the resulting mutated plasmid is used as a matrix for carrying out the second mutagenesis reaction in order to obtain the plasmid comprising the double mutation. When two mutations are separated by less than 5 amino acids, these two mutations are carried out

simultaneously with a single oligonucleotide which carries the two mutations.

The expression vector containing the mutated sequence is then used to transform a host cell which allows expression of the sequence of interest. The polypeptide produced is then isolated from the culture medium using conventional techniques which are well known to those skilled in the art, such as ethanol precipitation or ammonium sulfate precipitation, acid extraction, anion/cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography and lectin chromatography. Preferably, high performance liquid chromatography (HPLC) is used in the purification.

The purified polypeptide may be in various forms depending on the expression system used (secreted or nonsecreted protein) and the purification process. It may be in a denatured or nondenatured, monomeric or multimeric form. When it is in a denatured form, it is possible to return it to its open conformation according to the invention using the process described in Example 1. In order to obtain multimeric forms and in particular trimers, the purified polypeptide molecules must be placed in a medium in which the molecules are completely soluble, have no interactions with one another and preferably have no secondary structure. For this, detergents such as sodium dodecyl sulfate, N lauryl sarcosine, guanidinium chloride, urea, sodium thiocyanate or chaotropic agents may be used. The desired conditions may be promoted by using organic solvents or acids. Once this first condition is satisfied, the sample is placed in a dialysis cassette in order to remove some of the chaotropic agents, in such a way as to promote the interactions between the polypeptide monomers while maintaining sufficient solubility for the molecules. In a second step, the formation of the trimers having been promoted, the

sample is thoroughly dialyzed in a physiological medium which maintains the polypeptide solution or in suspension. Trimers of the polypeptide according to the invention, in an open conformation, are then obtained. Such a technique is described in detail in WO 00/08167.

Any expression vector conventionally used for expressing a recombinant protein may be used in the context of the present invention. This term therefore encompasses both "living" expression vectors, such as viruses and bacteria, and expression vectors of the plasmid type.

Use is preferably made of vectors in which the DNA sequence of the polypeptide according to the invention is under the control of a strong promoter which may be inducible or noninducible. By way of example of a promoter which may be used, mention may be made of the T7 RNA polymeraze promoter.

The expression vectors preferably include at least one selection marker. Such markers include, for example, dihydrofolate reductase or neomycin resistance, for culturing eukaryotic cells, and kanamycin, tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria.

By way of an expression vector which may be used in the context of the present invention, mention may be made of the plasmids pET28 (Novagen) or pBAD (Invitrogen) for example; and viral vectors such as: baculoviruses, pox viruses, in particular the pox viruses described in US patents 5,942,235, 5,756,103 and 5,990,091, or recombinant vaccinia viruses, in particular the recombinant viruses described in patents EP 83286, US 5,494,807 and US 5,762,938.

The site-directed mutagenesis is carried out according to the conventional techniques commonly employed by those skilled in the art, for example using the *Pfu* polymerase (Quick Change Mutagenesis Kit, Stratagene) or the bio rad mutagenesis kit. The mutations are confirmed by sequencing in the usual

manner. This type of method is described in detail in Maniatis et al. (Molecular cloning, a laboratory manual, cf. above).

In order to promote the expression and purification of the polypeptide, the latter may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. For example, a region containing additional amino acids, particularly charged amino acids, may be added to the N-terminal of the polypeptide in order to improve stability and persistence in the host cell.

Any host cell conventionally used in combination with the expression vectors described above may be used for the expression of the polypeptide.

By way of nonlimiting example, mention may be made of the E. coli cells BL21 (λDE3), HB101, Top 10, CAG 1139, Bacillus and eukaryotic cells such as CHO or Vero.

In the context of the present invention, the following expression vector/cell system: pET(Cer)/BL21LamdaDE3 or BL21lamdaDE3(RIL) will preferably be used.

The polypeptides of the present invention may be glycosylated or nonglycosylated, depending on the host employed in the procedure for production via the recombinant pathway. In addition, the polypeptides of the invention may also include an additional N-terminal methionine residue.

A subject of the present invention is also the conjugates comprising a polypeptide according to the invention and a carrier protein or a carrier peptide. The carrier protein (or peptide) reinforces the immunogenicity of the polypeptide according to the invention, in particular by increasing the production of specific antibodies. Said carrier protein (or peptide) preferably comprises one or more helper-T epitope(s). The term "helper-T epitope" is intended to

mean an amino acid chain which, in the context of one or more class II MHC molecules, activates helper T lymphocytes. According to an advantageous embodiment, the carrier protein (or peptide) used improves the water-solubility of the polypeptide according to the invention.

As a carrier protein, use may be made, for example, of phage surface proteins, such as the pIII or pVIII of the protein M13 phage, bacterial surface proteins, such as the LamB, OmpC, ompA, ompF and PhoE proteins of E. coli, the CotC or CotD protein of B. Subtilis, bacterial porines, such as porine P1 of Neisseria gonorrheae, porine P1 or P2 of H. influenzae B, the class I porine of N. meningitidis B or porine P40 of K pneumoniae, lipoproteins, such as OspA of B. bugdorfi, PspA of S. pneumoniae, TBP2 of N. meningitidis B, TraT of E. coli and also adhesin A of S. pneumoniae, and the heat shock proteins, such as Hsp65 or Hsp71 of M. tuberculosis or bovis, or Hin 47 of H. influenzae type B. Detoxified bacterial toxins, such as tetanus or diphtheria toxoid, the B subunit of cholera toxin, or the B subunit of endotoxin A of P. aeruginosa or exotoxin A of S. aureus, are also particularly suitable in the context of the present invention.

In the context of the present invention, carrier peptides which may be used include, for example, the p24E, p24N, p24H and p24M peptides described in WO 94/29339, and also the PADRE peptides as described by Del guercio et al (Vaccine (1997); vol 15/4, p441-448).

The carrier protein (or peptide) is linked to the N- or C-terminal end of the polypeptide according to the invention using any conjugation process which is well known to those skilled in the art. In addition, the sequence encoding the carrier protein (or peptide) may advantageously be fused to the sequence encoding the polypeptide according to the invention, and the

resulting sequence may be expressed in the form of a fusion protein using any conventional process. All the genetic engineering techniques which can be used to do this are described in Maniatis et al. Said conjugates can be isolated using any conventional purification process which is well known to those skilled in the art.

A subject of the present invention is also the DNA sequences encoding the polypeptides and the conjugates according to the invention, and also the expression vectors comprising said sequences and the host cells transformed with said vectors.

Rather than extracting and purifying the polypeptide or the conjugate expressed by the expression vector, it is often easier and sometimes more advantageous to use the expression vector itself in the vaccine according to the invention. A subject of the present invention is therefore also any expression vector as defined above.

A host cell as defined above which is transformed with such an expression vector is included in the context of the present invention.

A subject of the present invention is also the antibodies directed against the polypeptides and conjugates as described above. Such antibodies are prepared using the conventional techniques for producing polyclonal and monoclonal antibodies, which are well known to those skilled in the art.

These antibodies are particularly suitable for use in a passive immunization scheme.

A subject of the present invention is also vaccines which can be used for therapeutic and prophylactic purposes. The vaccines according to the present invention comprise at least one polypeptide, at least one conjugate or at least one expression vector as defined above, a pharmaceutically acceptable support or diluent and, optionally, an adjuvant.

The amount of polypeptide, conjugate or of vector in the vaccine according to the present invention depends on many parameters, as will be understood by those skilled in the art, such as the nature of the carrier protein, the vector used or the route of administration. A suitable amount is an amount such that a humoral immune response capable of neutralizing primary isolates of HIV is induced after administration of the latter. The amount of polypeptide to be administered is about 10 to 100 micrograms. The amount of conjugate to be administered will be deduced from the amounts indicated above, taking into account the MW of the carrier protein. The amount of expression vector to be administered is about 10 to 5000 micrograms in the case of a nonviral vector and about $10^{E}4$ to $10^{E}8$ TCID50 in the case of a viral vector.

The vaccines according to the present invention may also contain an adjuvant. Any pharmaceutically acceptable adjuvant or mixture of adjuvants may be used for this purpose. By way of example, mention may be made of aluminum salts, such as aluminum hydroxide or aluminum phosphate. Conventional auxiliary agents, such as wetting agents, fillers, emulsifiers, buffers etc., may also be added to the vaccine according to the invention.

The vaccines according to the present invention can be prepared using any conventional process which is known to those skilled in the art. Conventionally, the antigens according to the invention are mixed with a pharmaceutically acceptable support or diluent, such as water or phosphate buffered saline solution. The support or diluent will be selected as a function of the pharmaceutical form chosen, of the mode and route of administration and of pharmaceutical practice. Suitable supports or diluents and the requirements concerning pharmaceutical formulation are described in detail in Remington's Pharmaceutical Sciences, which represents a reference work in this field.

The vaccines mentioned above may be administered by any conventional route normally used in the field of vaccines, such as the parenteral (intravenous, intramuscular, subcutaneous, etc.) route. In the context of the present invention, intramuscular administration will preferably be used. Such an administration can advantageously be carried out in the muscles of the thigh or of the arm. Administration via the nasal, oral, vaginal or rectal mucous membrane route may also be recommended in the context of the present invention. The administration can be carried out by administering a single dose or repeat doses, for example on D0, at 1 month, at 3 months, at 6 months and at 12 months. Injections on D0, at 1 month and at 3 months, with a booster, the frequency of which may be easily determined by the treating physician, will preferably be used.

The vaccine according to the present invention may advantageously be administered according to a posology scheme comprising the co-administration of an expression vector according to the invention and of a polypeptide according to the invention, or according to a prime-boost scheme in which the vector according to the invention is administered first and the polypeptide is administered as a booster injection. In these two posology schemes, the expression vector according to the invention may be replaced with any expression vector expressing one or more HIV antigens or epitopes other than the polypeptide according to the invention, and in particular with an ALVAC or NYVAC vector.

The present invention is also understood to cover a polypeptide, conjugate or a vector as defined above and the vaccine containing these compounds, for their use in inducing antibodies which neutralize primary isolates of HIV.

The applicant has demonstrated, surprisingly, that the polypeptide according to the invention is capable, after administration, of inducing antibodies

which are capable of neutralizing primary isolates of HIV. These antigens therefore represent valuable candidates for developing a vaccine which can be used for the protection and/or treatment of a great number, or even all, of the individuals at risk or infected with HIV.

The present invention therefore also relates to a method for inducing an immune response in a host individual, including humans, comprising the administration of a vaccine according to the invention. The term "an immune response" is intended to mean a response comprising the production of antibodies directed specifically against the polypeptide according to the invention. The production of specific antibodies can be easily determined using conventional techniques which are well known to those skilled in the art, such as ELISA, IRA or Western blot.

A subject of the invention is also a diagnostic method comprising bringing a polypeptide according to the invention into contact with a biological sample and detecting the antibody/polypeptide complexes formed.

The polypeptide according to the present invention can in fact be used in an ELISA assay to detect the anti-gp41 antibodies which are present in the serum of individuals. The polypeptide according to the present invention is therefore useful as a diagnostic tool since the presence of anti-gp41 antibodies is a reliable marker for infection with HIV.

In this case, the polypeptide according to the invention is deposited onto an ELISA plate, and then brought into contact with serial dilutions of the serum of the patient to be tested and, finally, brought into contact with an anti-human antibody linked to an enzyme. The anti-human antibody/anti-gp41 antibody/polypeptide complex thus formed is then detected by colorimetry.

The present invention will be described in greater detail in the examples which follow, with reference to the attached figures in which:

Figure 1 is a schematic representation of the phenomenon of conformational change of gp41 which precedes the fusion of the cell and viral membranes.

Figure 2 gives the complete sequence of gp41 LAI in which (______) represents the fusion peptide and (-------) represents the transmembrane domaine.

Figure 3 gives the sequence of the polypeptide which derives from the gp41 LAI protein which is used as the starting product in the examples provided.

Figures 4 and 5 give a schematic representation of regions 1 to 4; Figure 4 summarizes the functions of the mutations envisaged. The numbers given between brackets refer to the regions identified, "promote the interactions (3-12)" meaning that the interactions between region 3 and regions 1 and 2 are promoted.

The examples described below are given purely by way of illustration of the invention and cannot in any way be considered as limiting the scope of the latter.

<u>Example 1</u>: Preparation of various polypeptides according to the invention

1- cloning the sequence of Figure 3 into an expression vector

The DNA sequence encoding the polypeptide identified in Figure 3 was cloned into an inducible expression system.

The vector used is pET-CER, which is constructed from the vector pET28 from Novagen. The commercial vector pET28c was amplified by PCR using 2 primers located on either side of the region corresponding to the origin F1, in such a way that the amplified product corresponds to virtually the entire vector of origin, less the region comprising the origin F1. The unique AscI and PacI restriction sites are provided,

respectively, by the two primers which have been used for the amplification. In parallel, the CER fragment is amplified using two primers which make it possible to obtain this fragment bordered by AscI and PacI sites.

The CER fragment and vector are digested with the AscI and PacI enzymes and then ligated to one another. This vector in particular comprises an expression cassette under the control of the T7 promoter, a polylinker downstream of the T7 promoter, for cloning the gene of interest, the CER fragment located downstream of the polylinker, making it possible to decrease the multimerization of the plasmids, a T7 term transcription terminator and the kanamycin resistance gene.

Positive regulation of the promoter is obtained in the presence of T7 RNA polymeraze.

2- Site-directed mutagenesis

The site-directed mutagenesis for producing the mutated polypeptides according to the invention is carried out using the QuickChange site-directed mutagenesis kit from Stratagene.

For each mutation, two mutagenesis oligonucleotides which border the amino acid to be mutated are defined. For example, for the R51A mutation, the following oligonucleotides are used:

	R
Reference sequence	**ctg gct gtg gaa aga tac cta aag gat**
	A
5′ oligonucleotide	**ctg gct gtg gaa gca tac cta aag gat**
3′ oligonucleotide	**atc ctt tag gta tgc ttc cac agc cag**

The 2 oligonucleotides will hybridize to the same sequence on the complementary strands of the plasmid containing the sequence to be mutated. The mutation is located at the center of the oligonucleotides and it is bordered by 12 nucleotides on each side.

The mutagenesis reaction is carried out on the plasmid of Example 1 under the following conditions: a mixture containing: 5 µl 10X reaction buffer; 1 µl plasmid to be mutated, 100 ng/µl; 1 µl 5'Oligo (125ng/µl); 1 µl 3'Oligo (125ng/µl); 1µl 10 mM dNTP Mix; 40µl UF H_2O; and 1µl Pyrococcus furiosus heat-stable polymeraze, 2.5U/µl, is subjected to a PCR according to the cycles defined below: 95°C, 30"; 95°C, 30"; 55°C, 1'; 68°C, 2'/kpb of plasmid; 12 cycles; temperature at the end of the reaction: 20°C.

Using the protocol above, the various mutants identified in Table 1 were prepared.

3- Expression

The expression of the plasmids derived from step 2 above is carried out in E. coli. To do this, a modified E. coli strain is used: BL21 IRLλDE3.

This strain is enriched in rare tRNAs (ARG, ILE, LEU); it contains the gene encoding T7 RNA polymerase, which gene is under the control of the *lac* UV5 promoter which can be induced by adding IPTG at a concentration of 1 mM.

Initially, the strain is transformed with the mutated plasmid according to the protocol comprising the following steps: 3 colonies are subcultured in 10 ml of LB+ANTIBIOTIC; they are incubated overnight at 37°C; the preculture is re-seeded in 15 ml of LB+ANTIBIOTIC at 1:100; it is allowed to grow until an OD600 of 0.5 is obtained; 1 ml is removed in order to verify the OD600; 7 ml are removed for the noninduced sample; the other 7 ml are induced with 1 mM of IPTG and induction carried out for 3H at 37°C.

The same protocol was carried out on several liters of culture in order to produce a large amount of bacteria for purifying the mutated polypeptide.

4- Purification

The cell pellet made up of the bacteria harvested from one liter of culture medium is thawed and taken up in 2x 100 ml of 30mM Tris buffer at pH8 in the presence of

a protease inhibitor (Pefabloc, Interchim) at a concentration of 100 µM. Lysozyme is added at the concentration of 100 µg/ml and the mixture is incubated for 30 minutes at room temperature. The cells are then ruptured by sonication (4 cycles of two minutes) with an approximate power of 150 watts. The gp41 is in the form of inclusion bodies. They are washed in a PBS-0.05% tween 20 buffer at 4°C and centrifuged for 15 minutes at 10,000 g. After removing the supernatant, the centrifugation pellet composed essentially of the inclusion bodies is solubilized over one hour at room temperature with gentle stirring in the presence of 50 ml of CAPS buffer at pH 10.4 containing 3% of N-lauryl sarcosine.

The solubilized fraction is then dialyzed at 4°C against a 30 mM Tris buffer at pH 8 containing 8 M urea (5 bars), filtered through a filter with a porosity of 0.45 µm and then loaded onto a 1 ml high Hi-Trap column (Pharmacia). These affinity chromatography supports chelate nickel atoms to which the histidine residues of the C-terminal end of the protein attach.

After washing, the protein is eluted in the 30 mM Tris buffer at pH 8 containing 8M urea and 500 mM imidazole. The eluted fractions are dialyzed in a 30 mM Tris buffer at pH8 containing 8M urea, but with no imidazole, and with decreasing amounts of urea, ranging down to 2M. This technique makes it possible to purify all the mutant or native gp41 molecules in the presence or absence of the fusion peptide.

Example 2: Immunogenicity and induction of neutralizing antibodies

The immunogen tested in this example corresponds to a polypeptide of sequence SEQ ID No. 2 into which the mutation I101D has been introduced by site-directed mutagenesis, and which comprises, at the C-terminal, a sequence of histidines in order to facilitate the purification thereof. The preparation of

the immunogen was carried out according to the methods described in the preceding examples.

Groups of 5 guinea-pigs were immunized three times by intramuscular injection (into the thigh, in the *biceps femoris* muscle) 3 weeks apart (days 1, 22 and 43) with 20 µg per dose of native gp41 or of polypeptide I101D in the presence of 6 mg of aluminum phosphate. The immunogen was administered in a volume of 0.5 ml, i.e. 0.25 ml per thigh. Serum samples were taken on D1, D43 and D57.

The individual immune sera (D43) and the mixtures of preimmune sera (D1) from each group were tested by ELISA for their IgG antibody titers induced against native gp41 and against gp160 MN/LAI-2.

The individual immune sera (D57) and the mixtures of preimmune sera (D1) from two groups were tested for their sero-neutralizing activity with respect to the primary HIV-1 isolate Bx08.

The results obtained show that the polypeptide according to the present invention is as immunogenic as native gp41.

In addition, the neutralization assay of C. Moog et al. showed that the polypeptide according to the invention induces neutralizing antibodies (% reduction > 10-fold).

SEQUENCE LISTING

<110> AVENTIS PASTEUR

<120> POLYPEPTIDE INDUCING ANTIBODIES WHICH NEUTRALIZE HIV

<130> PM0101

<140>
<141>

<160> 2

<170> PatentIn Ver. 2.1

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<211> 344
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<213> Human immunodeficiency virus type 1

<220>
<221> PEPTIDE
<222> (1)..(344)
<223> gp41 LAI protein

<400> 1
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gikqlqaril averylkdqq llgiwgcsgk licttavpwn aswsnksleq iwnhttwmew 120
dreinnytsl ihslieesqn qqekneqell eldkwaslwn wfnitnwlwy iklfimivgg 180
lvglrivfav lsivnrvrqg ysplsfqthl ptprgpdrpe gieeeggerd rdrsirlvng 240
slaliwddlr slclfsyhrl rdlllivtri vellgrrcwe alkywwnllq vwselknsav 300
sllnataiav aegtdrviev vqgacrairh iprrirqgle rill                  344
```

<210> 2
<211> 177
<212>
<213> Human immunodeficiency virus type 1

<220>
<221> PEPTIDE
<222> (1)..(177)
<223> polypeptide derived from gp41 LAI

<400> 2
```
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iwgcsgklic ttavpwnasw snksleqiwn nmtwmewdre innytslihs lieesqnqqe 120
kneqelleld kwaslwnwfn itnwlwyikn rvrqgyspls fqthlptprg pdrpegi    177
```

CLAIMS

1. A polypeptide capable of forming a structure corresponding to or mimicking the intermediate state of gp41, comprising at least one mutation selected from the group consisting of: I101D or S.

2. The polypeptide as claimed in claim 1, of sequence SEQ ID No. 2 and comprising at least one mutation selected from the group consisting of: I101D or S.

3. The polypeptide as claimed in claim 1 or 2, comprising at least one other mutation selected from the group comprising: G13A, L, M, I, W or K; Q17A or E; Q18A or E; A24Q, E, S or R; Q28A; T35I or L; V36Q or E; W37S or D; G38A, V, L, I, M or E; Q39A, V, L, I, M or E; K40E, A, V, L, I or M; Q41A, V, L, I, M or E; Q43A, V, L, I, M or E; L47A or D; V49I or L; R51A, N or E; Q56I; C64S; C70S; or L; W94D; D98A, V, L, I, M or K; R99A, N or E; Y104M or E; I108D; Q119A, V, L, I, M, S, N or R; E120A; K121A; E123A; E125A; R153N or A and R173N or A.

4. The polypeptide as claimed in any one of claims 1 to 3, comprising the following mutations: T35I + Q28I + I101D; T35I + Q28I + I101D + Q119N; I101D + I108D + Q131N + W37A; I101D + I108D + Q142N + L126D; W37A + I101D + I108D + Q119N; or I101D + I108D + Q119N + L126D.

5. A conjugate comprising a polypeptide as claimed in one of claims 1 to 4, conjugated to a carrier protein or peptide.

6. A DNA sequence encoding a polypeptide as claimed in any one of claims 1 to 4 or encoding a conjugate as claimed in claim 5.

7. An expression vector comprising the DNA sequence as claimed in claim 6.

8. A host cell containing the vector as claimed in claim 7.

9. A process for preparing a polypeptide as claimed in any one of claims 1 to 5, comprising the expression of said polypeptide using a host cell as defined in claim 8.

10. A vaccine against HIV, comprising at least one polypeptide as claimed in one of claims 1 to 4, at least one conjugate as claimed in claim 5 or at least one expression vector as claimed in claim 7, a pharmaceutically acceptable support and, optionally, an adjuvant.

11. The use of a polypeptide as claimed in any one of claims 1 to 4, for preparing a medicinal product for inducing antibodies which neutralize primary isolates of HIV.

Figure 1

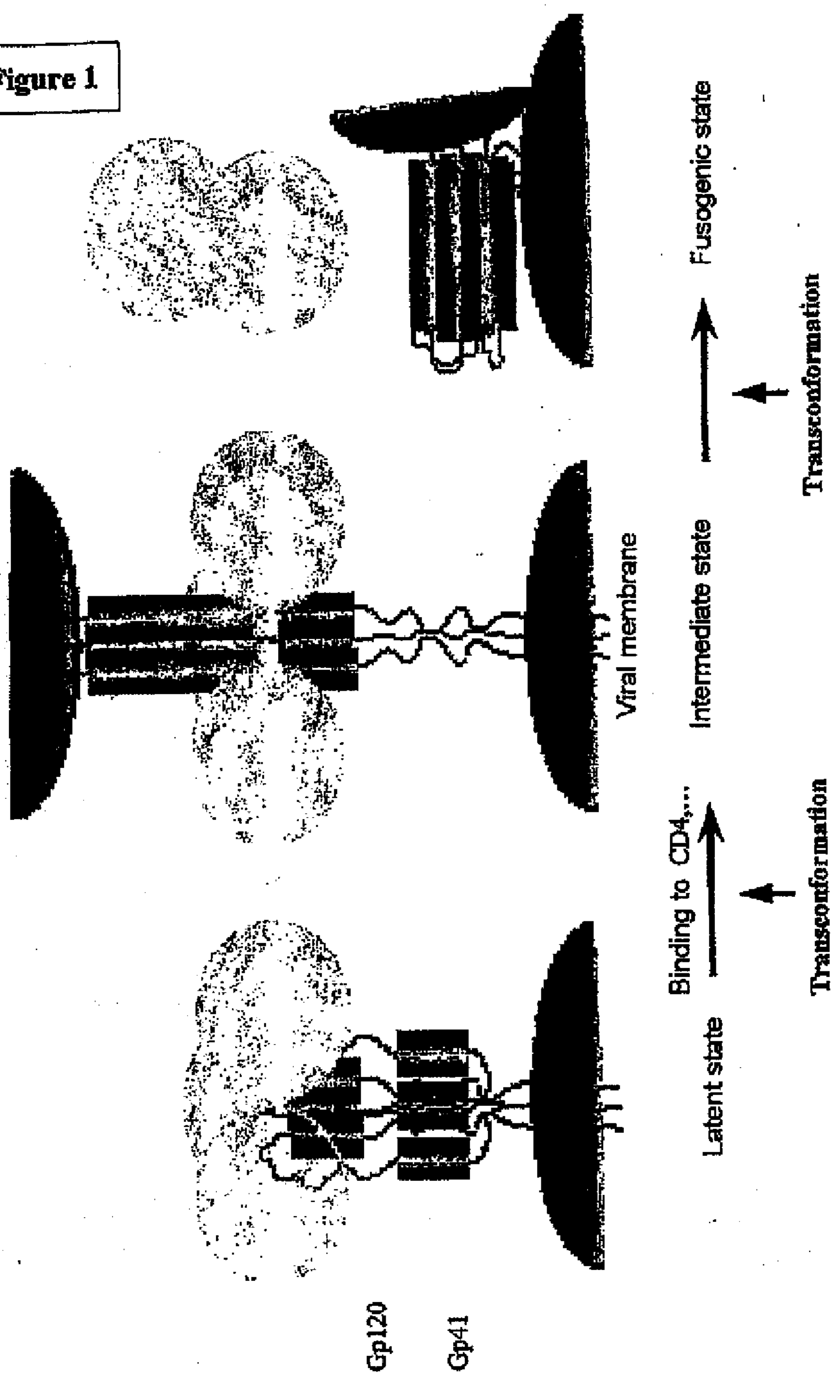

Gp120
Gp41
Latent state
Binding to CD4,...
Transconformation
Viral membrane
Intermediate state
Transconformation
Fusogenic state

Figure 2 : **Complete sequence of the gp41 LAI protein**

```
 avgigalfl gflgaagstm gaasmtltvq arqllsgivq qqnnllraie 49
aqqhllqltv wgikqlqari laverylkdq qllgiwgcsg klicttavpw 99
naswsnksle qiwnnmtwme wdreinnyts lihslieesq nqqekneqel 149
leldkwaslw nwfnitnwlw yiklfimivg glvglrivfa vlsivnrvrq 199
gysplsfqth lptprgpdrp egieeegger drdrsirlvn gslaliwddl 249
rslclfsyhr lrdlllivtr ivellgrrcw ealkywwnll qvwselknsa 299
vsllnataia vaegtdrvie vvqgacrair hiprrirqgl erill      344
```

_____: **Fusion peptide (AA 1-23)**
-----: **Transmembrane domain (AA173-194)**

Figure 3: **Sequence of the polypeptide which derives from the gp41 LAI protein used as the starting product in the examples.**

```
    mtltvq arqllsgivq qqnnllraie aqqhllqltv wgikqlqari 46
laverylkdq qllgiwgcsg klicttavpw naswsnksle qiwnnmtwme 96
wdreinnyts lihslieesq nqqekneqel leldkwaslw nwfnitnwlw 146
yiknrvrqgy splsfqthlp tprgpdrpeg i                     177
```

Figure 4

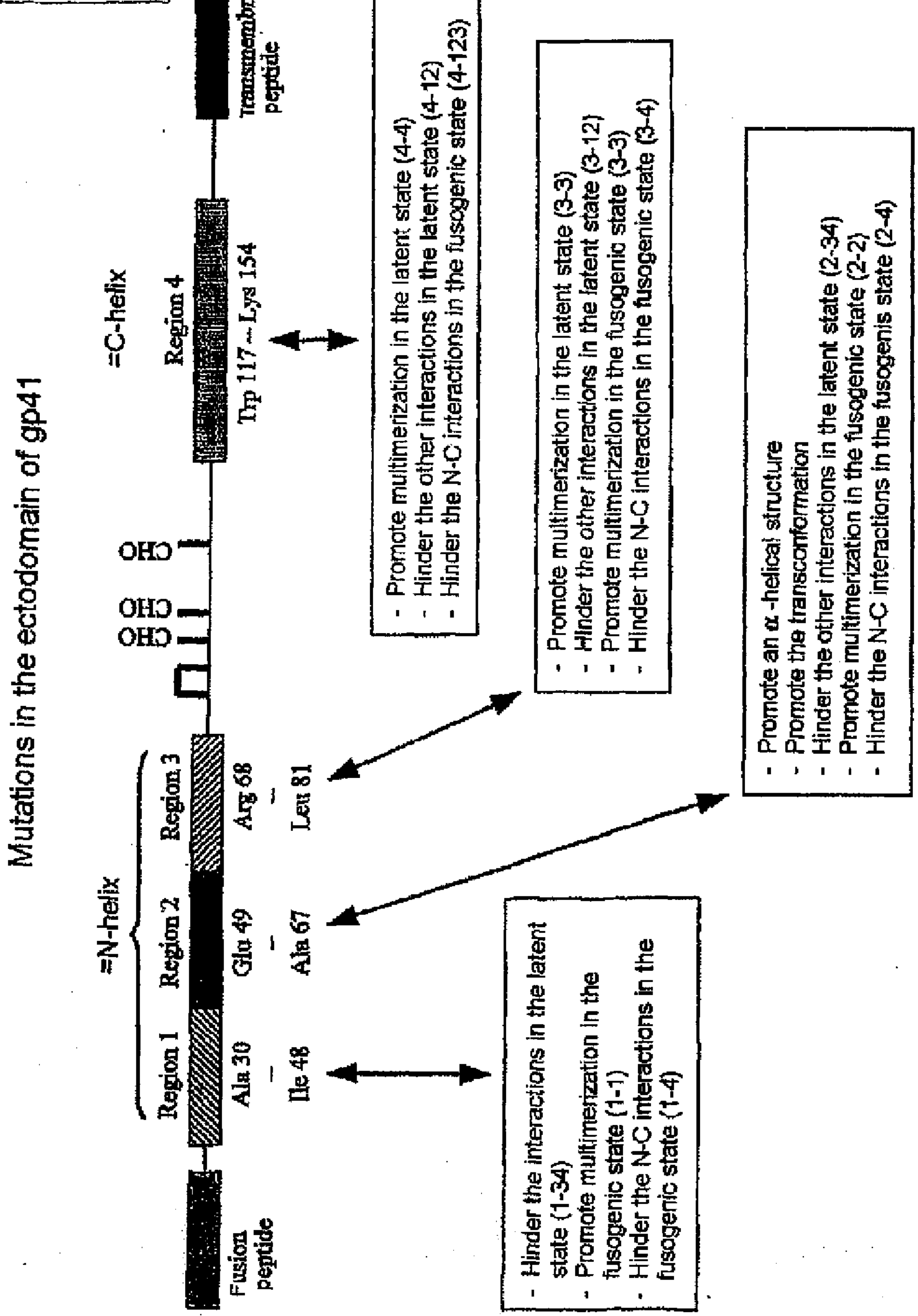
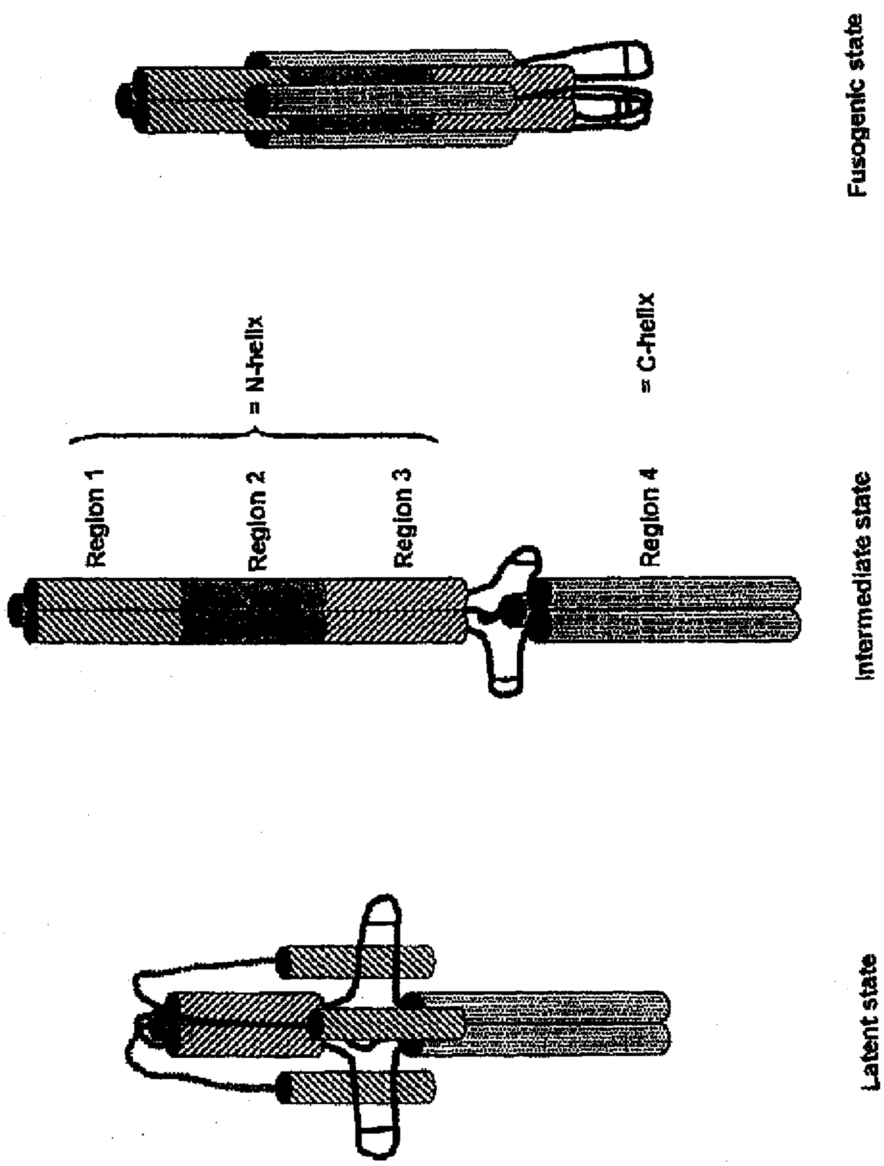

Mutations in the ectodomain of gp41
=N-helix
=C-helix
Fusion peptide
Region 1
Region 2
Region 3
Region 4
CHO
CHO
CHO
Transmembrane peptide
Ala 30 – Ile 48
Glu 49 – Ala 67
Arg 68 – Leu 81
Trp 117 – Lys 154
- Hinder the interactions in the latent state (1-34)
- Promote multimerization in the fusogenic state (1-1)
- Hinder the N-C interactions in the fusogenic state (1-4)
- Promote an α -helical structure
- Promote the transconformation
- Hinder the other interactions in the latent state (2-34)
- Promote multimerization in the fusogenic state (2-2)
- Hinder the N-C interactions in the fusogenis state (2-4)
- Promote multimerization in the latent state (3-3)
- Hinder the other interactions in the latent state (3-12)
- Promote multimerization in the fusogenic state (3-3)
- Hinder the N-C interactions in the fusogenic state (3-4)
- Promote multimerization in the latent state (4-4)
- Hinder the other interactions in the latent state (4-12)
- Hinder the N-C interactions in the fusogenic state (4-123)

Figure 5


Fusogenic state
Region 1
Region 2
Region 3
= N-helix
Region 4
= C-helix
Intermediate state
Latent state